

COMPARATIVE STUDY OF THE DEVELOPMENT OF
TRICHURUS SPIRALIS AND STYSANUS STEM-
ONITES.

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(WITH PLATES XXIII AND XXIV)

TRICHURUS SPIRALIS.

DURING the autumn of 1898 a hyphomycete, closely resembling the members of the genus *Stysanus*, was found on decaying wood brought into the laboratory of Cornell University. The principal difference between this fungus and the species of *Stysanus* lies in the fact that the capitulum of the former is densely beset with long, tortuous, sterile threads, recalling at first sight the spiral setae surrounding the perithecia of some species of *Chaetomium*. This character shows the plant to be closely related to *Trichurus cylindricus* Clements & Shear.¹ In the following pages I will refer to it as *Trichurus spiralis*, leaving a discussion of its history and nomenclature until the end of this paper. The plant was obtained in pure culture, and grown on agar and on bean stems, in order to study the development of the sporophore, and to determine, if possible, whether any other forms of fructification existed in the life cycle of the fungus.

The perfect sporophores of *Trichurus spiralis*, growing on agar or on bean stems, appear as small fluffy heads, which are either linear or elongated oblong, and obtuse or pointed at the summit. In agar they appear in small clusters of several heads near the center of the colony, but when grown on bean stems they arise over the entire surface of the substratum. Usually the sporophores are simple, but not infrequently they are branched near the base, and bear several smaller heads, or the head itself may be divided above. These rather abnormal forms are probably caused by an abundance of nutriment. While

¹ Rept. Bot. Surv. Nebraska 4:7. 1896.

fresh and growing the heads are blackish gray, but when dry they assume a copper tint, or rather a color resembling the so-called purple-brown of the dark-spored agarics. The heads are supported on thin black stalks, which are usually longer than the head itself. The entire sporophore varies in length from 0.75 to 2^{mm}; sometimes under favorable conditions larger heads are formed on bean stems, but this would scarcely occur in nature.

The microscopic structure of the sporophore is shown in *fig. 1*. The stipe is made up of many brown septate threads, growing closely together in a strict fascicle. Near their summit the threads send out many short bottle-shaped basidia, which, without being attenuated to a distinct sterigma, bear long chains of spores. Among the spore-chains there arise many long tortuous threads, which form the distinguishing characteristic of this plant. The threads are brown near the base, becoming hyaline at the tips, and give the head the characteristic flocculent appearance. From the base of the stipe numerous threads radiate in all directions. These threads are septate like those of the stipe. They are brown near their origin, but terminate in hyaline growing points. They will be more fully treated below.

The ripe spores of this plant are oval to oblong, with rounded or pointed ends (*fig. 2*). In a single preparation, however, so many variations from this form are found that this description cannot be strictly applied. Sometimes they are even and quite regular in shape; then again one or both ends may be pointed lemon-form; or the entire spore may be more or less inequilateral and irregular. Under the microscope they are dilutely yellowish-brown. The differentiation between the wall and content cannot be recognized easily, the entire spore appearing as a homogeneous disk surrounded by a dark refractive ring. With high powers and careful focusing the wall and protoplasm can be made out. The spores are about 5-6 μ in length, by 2.5-3 μ in diameter.

When these spores are sown on agar, germination begins immediately, and the spore swells until it is nearly twice its

original size. At the same time the refractive ring disappears, and the protoplasm becomes distinctly granular and vacuolate. The wall on one side then seems to undergo a change. It becomes thin and delicate, until it can scarcely be distinguished, finally bulging out in the form of a short stout germ tube, whose diameter nearly equals the length of the spore (*fig. 3*). The rest of the spore membrane remains as a sort of cap over the end of the germ-tube. The entire protoplasm of the spore is used in the formation of this first germ tube. The formation of a second similar one would seem impossible, and was never observed. When this primary germ tube has attained a length about equal to the diameter of the spore, it sends out two branches from opposite sides of its broad terminal portion. This formation of a primary tube before the appearance of true hyphae is not unlike the mode of germination of the gonidia of *Completozia complens* by means of a proembryo, as described by Professor Atkinson;² or it may be compared to the extrusion of the endospore in *Ceratostoma brevirostre* before the formation of a true mycelium, as described and figured by Miss Nichols.³ The first mycelial branches are thin, about 3-4 μ in diameter, and elongate rapidly, usually in opposite directions (*fig. 4*). The protoplasm in the older parts of the tubes is rather coarsely granular, with many irregular indistinct vacuoles. Near the growing portion it is homogenous, and at the tips perfectly hyaline. Numerous lateral branches soon arise in a very irregular manner from the main hyphae, being directed at a more or less acute angle to them (*fig. 5*). The branches have the same general appearance as the threads from which they arise, and equal them in diameter, and all hyphae of the mycelium contain numerous septa.

At the end of four days the mycelium has developed a well defined compact colony about 5^{mm} in diameter, with an even margin. The colony consists of a mass of threads so wedged

² Damping-off. Cornell Univ. Exp. Sta. Bull. 94: 233-272. *pls. 6*. 1895.

³ The morphology and development of certain pyrenomycetous fungi. BOT. GAZ. 22: 301-328. *pls. 3*. 1896.

in together that they form a thick mat. None of the vegetative hyphae grow above the agar, so that the colony never presents a flocculent appearance.

If at this time one of the colonies is lifted out of the agar and examined under the microscope, its surface will be found to be dotted with small peculiar sporophores, resembling very much the conidial fructification of *Penicillium*, or still more, perhaps, on account of the slight coloration of the spores, the form genus *Haplographium* (*figs. 6, 7*). These gonidiophores show great variability. Those first formed consist of a single erect hypha bearing a chain of gonidia at its summit. Others have three or four branches, each with a chain of gonidia. In the older parts of the mycelium, toward the center of the colony, these small gonidiophores become more and more complex (*fig. 7*). Their branches divide and subdivide until it is impossible to represent them. In some of the gonidiophores a sterile branch takes the place of a basidium (*fig. 7*), and later, when the colony is a little older, all the gonidiophores bear long-curved setae, many times longer than the entire fruiting head, so that it sometimes seems as if a small stalk had spent all its effort in producing one of these enormous curved setae.

In these sterile threads, intermingled with the chains of gonidia, one sees a resemblance between the *Penicillium* form and the perfect sporophores. In time, still greater similarity appears. The heads formed near the center of the colony begin to assume a different appearance. The stalks are considerably elongated, and become brown and septate before the head of gonidia is formed (*figs. 8, 9*). Often two or three grow near together, forming a distinct stipe like that of the perfect sporophore in every respect except size. The stalk hyphae do not remain simple, but send out branches from their lower cells. These branches grow upward and soon attain a diameter equal to that of the stalk. The manner of origin of these branches can be seen best in the more simple sporophores. They are usually sent out from the lower cells, always originating immediately below a septum (*figs. 9, 10 a*). *Fig. 8* shows a small

sporophore which originated from a single erect hypha. The two lower cells have sent out branches, which, together with the original hypha, have formed a stalk bearing a small head of gonidia at its summit. The young branches are hyaline at the tips, but brown in the older portions. They soon become merged with the other hyphae forming the stipe.

In the more complex forms of the small gonidiophores there is seen an approach to the perfect sporophores of *Trichurus*. In fact, they are identical with the latter both in structure and manner of formation, differing only in the number of hyphae which go to make up the stipe and head. It is easy to form a complete series, showing all gradations from the simplest hypha bearing a single chain of gonidia to the most complex sporophore with several hundred spore-chains.

At a time varying from four to six days after sowing, the vegetative hyphae have formed a dense mat of mycelium in the medium in which they are growing. At the center of the colonies the threads become so numerous and interwoven that they form almost a stroma. From this stroma-like mat the perfect sporophores arise, either singly or in small clusters. They first appear as stout fascicles of hyphae, which arise perpendicular to the substratum. The growing points of the threads are hyaline, but in the older parts of the bundle they assume the characteristic brown color of the stipe (*fig. 18*, of *Stysanus stemonites*).⁴ The threads branch in the manner described above, the branches remaining closely appressed to the bundle and growing with it. During longitudinal growth the central threads are in advance of the peripheral hyphae, giving the bundle a linear, pointed form. Before the bundle has completed its growth, the individual hyphae begin to send out small, curved branches or basidia at some distance from their growing points. The basidia are thus formed in centripetal order. Those first formed begin to bear chains of spores before the bundle or stipe has completed

⁴The formation of the stipe and head in the two forms, *Trichurus spiralis* and *Stysanus stemonites*, is exactly alike, and it was not considered necessary to represent this stage more than once. *Figs. 18* and *19* are from *Stysanus*, but they will answer equally well for *Trichurus*.

its growth. Finally, the threads themselves cease to elongate, their ends become rounded and enlarged, and begin bearing chains of spores like the basidia (*fig. 19*). Thus the hyphae of the stalk finally terminate in basidia. Septa are formed in the threads of the stipe somewhat later than the basidia, but their formation progresses in the same order, from the base upwards.

The basidia, as has been said, appear as short, curved, rather blunt branches. Like all growing parts they are at first hyaline, and their tips remain colorless throughout. They elongate slightly, resembling a short bottle in shape. The first spore begins to appear at the end of the basidium as a globular swelling which increases in diameter until it has reached the normal size of a mature spore. Before the first spore has completed its growth the tip of the basidium just beneath it enlarges, and a second spore appears below the first. The tip of the basidium continues thus to elongate and to cut off spores until a long chain is formed, or until the nutriment is exhausted. The exact number of gonidia thus formed in a chain cannot be well determined, as they are very much tangled in the head, and when mounted in water they break apart instantly. Sometimes, however, a gonidiophore which has sunk down into the agar is found with some of its chains partially unbroken. In these chains fifteen to eighteen spores were often counted, but the total number is probably much greater. The individual spores are connected by a short isthmus which can be distinguished with difficulty, in the mature chains, but when spores lying on the agar have begun to germinate, it can be distinctly made out as a short tube connecting the swollen spores.

The sterile threads push out from the heads soon after the formation of gonidia has begun. When first seen they are straight, being directed at right angles to the long axis of the head. They are at first hyaline, but as they grow longer they become brown. As the setae grow they twist about in various directions, but never become branched. Septa are formed at short intervals in their older parts.

In describing the perfect sporophore of this plant attention

was called to the threads which radiate evenly from the base of the stipe (*fig. 1*). From the position of these hyphae near the surface of the nutrient medium, and from the fact that they generally terminate in delicate growing points, and are not much interwoven with the threads of the mycelium, it seems that they do not originate from the mycelium, but grow out from the base of the stipe. A study of their origin showed this to be the case. Some of the branches sent out from the hyphae of the young gonidiophore are directed downwards toward the substratum (*fig. 9, b*). These branches were found on many occasions and in all stages of development, from those which had begun to others which had grown to a considerable length. It is interesting to note that those branches destined to grow upward constantly originated just below a septum, while those growing toward the substratum always began above a cross-wall. In many cases observed this was invariably true. Without doubt these radiating branches serve to support the sporophore for, as it often starts from a comparatively small bundle of hyphae, the large head of gonidia with its sterile threads would probably bear it down if it were not strengthened by further support.

The life cycle of this plant seems thus completed. Although many cultures were made in different media no perfect form was obtained, and indeed it seems probable that among some of the more highly developed Hyphomycetes like *Trichurus* the perfect form has been lost even if it ever existed.

STYSANUS STEMONITES.

Stysanus stemonites is a common form of the *Phæostilbeæ*⁵ found growing on decaying wood and bark of all kinds, on which its small sporophores form gray patches of varying extent. On account of its great resemblance to *Trichurus* it was grown in a series of cultures parallel with those of the latter fungus, especially as the thought suggested itself that perhaps the sterile bristles might be merely an abnormal production of *Stysanus*. The cultures for this purpose were obtained from

⁵Sacc. Syl. Fung. 4: 603.

stock tubes in the laboratory. It is needless to say that throughout the cultures the differences between the two forms remained constant. The difference in the size of the two plants alone would be sufficient to remove all doubt as to their specific distinctness. In the course of the cultures, however, so many points of resemblance between the two plants were found, including a small form of fructification new to *Stysanus*, that it seems worth while to mention them here.

The spores of *Stysanus stemonites* are in general oblong or lemon-shaped, but, as in *Trichurus*, they are more or less irregular, and extremely variable in shape and size (*fig. 11*). They are usually more pointed than in *Trichurus*. Under the microscope the spores are dilutely olive-brown, almost hyaline, having the usual dark refractive line around the border. They measure from 6-7 μ in length by 3-4 μ in diameter, but vary outside of these limits.

Germination of the spores begins immediately after sowing. The process takes place exactly as was described for *Trichurus*, that is by the formation of a thick primary germ tube (*fig. 12*). After eighteen to nineteen hours the mycelium has reached the stage of development shown in *fig. 14*. The tubes are rather slender, rarely exceeding 3-4 μ in diameter. The protoplasm is somewhat indistinctly granular and vacuolate.

The colonies formed by the mycelium are smaller than those of *Trichurus* of the same age. The hyphae do not show a great tendency to spread out in the agar but remain within a small compass. At the end of four days growth the colonies are scarcely more than 3-4^{mm} in diameter. The mycelium branches abundantly and irregularly, forming a dense mat whose center is raised slightly above the agar. The center is even more stroma-like than in *Trichurus*. The colonies soon become covered with a gray gonidial growth which extends almost to their margin. This growth consists of Penicillium-like gonidiophores resembling those described for *Trichurus*. In this case, however they are much more abundant, thus differing in a marked manner from *Trichurus*, in which they are never sufficiently numerous

to change the external appearance of the colony (*figs. 15, 16, 17*). The gonidiophores themselves, on the other hand, are very much alike in the two plants, as *figs. 15, 16* and *17* will show. In *Stysanus* also we can find a complete series showing all gradations between these small gonidiophores and the perfect sporophores.

The mature sporophores are small, rarely attaining a height of 0.75^{mm} . They arise either singly from the center of the colony, or several arise together from a stroma-like base. The stipe elongates rapidly, and soon begins to bear basidia and chains of gonidia which remain together in a tangled head so long as they are dry, but when placed in water they break apart instantly. Such sporophores from which most of the gonidia have fallen away are shown in *figs. 20* and *21*.

CONCLUSION.

From this study there can be no doubt of the close relationship of *Trichurus spiralis* and *Stysanus stemonites*. Not only are the perfect forms similar to each other, but they agree closely in their entire development, which may be summed up as follows. The spores germinate in a peculiar manner by means of a stout primary germ tube or proembryo. The mycelium forms a small, compact colony, from which there arises a gonidial fructification very different from the normal fructification of the plants. The normal fruit consists of a bundle of hyphae bearing basidia and chains of gonidia at the summit. All gradations between the simple sporophores and the compound heads exist. The characters which distinguish these two plants from each other are the presence of tortuous sterile threads in the head of *Trichurus* and the difference in the size of the plants and spores. Among these low forms these characters are, perhaps, sufficient for generic separation. The plant treated in the first part of this paper has provisionally been referred to the genus *Trichurus* Clements & Shear. *Trichurus* was characterized by them thus: "As in *Stysanus*, but the capitulum densely beset with long strict bristles." The present plant, however, differs from this

description in the possession of curved setae. In order to include this plant in the genus, the generic description would have to be recast as follows: Stroma erect, cylindrical, dark colored, rigid; conidia borne in a loose, oblong head, ovoid or lemon-form, sub-hyaline; head beset with simple or branched, straight or curved, sterile threads or setae. The genus would include the two following species:

TRICHURUS CYLINDRICUS Clements & Shear. Rept. Bot. Surv. Nebraska 4; 7. 1896.

Trichurus spiralis, n. sp.—Sporophores 0.75—3^{mm} high, solitary or in blackish-gray patches: stipe simple or branched, black, composed of many brown septate threads: capitulum shorter than the stipe, cylindrical, obtuse or pointed at the apex, often divided into several smaller heads, beset with simple, brown, septate, tortuous bristles, which are hyaline at the end: gonidia catenulate, oval to oblong, with rounded or pointed ends, often inequilateral or lemon-form, dilutely yellowish-brown, 5–6 μ by 2.5–3 μ .

On decaying wood, raspberry canes, and on an insect pupa. The plant is easily recognized by the long brown sterile threads in the capitulum.

As was stated at the beginning of this paper, *T. spiralis* was obtained from decaying wood in the autumn of 1898. The same plant had been previously collected here by Professor Atkinson on raspberry canes. Specimens from cultures of this former collection were preserved, and although spores from the older material failed to germinate, the plant was easily identified as being the same as that here described.

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EXPLANATION OF PLATES XXIII AND XXIV.

PLATE XXIII. *Trichurus spiralis*.

- FIG. 1. Perfect sporophore.
 FIG. 2. Spores.
 FIG. 3. Germinating spores.
 FIG. 4. Same forming mycelium.
 FIG. 5. Young mycelium.
 FIGS. 6–7. Penicillium-fruits.
 FIGS. 8–10. Small sporophores; 9*b*, descending branch; 9*a* and 10*a*, ascending branches.

PLATE XXIV. Stysanus stemonites.

- FIG. 11. Spores.
FIGS. 12-13. Germinating spores.
FIG. 14. Young mycelium.
FIGS. 15-17. Penicillium-fruits.
FIG. 18. Growing sporophore.
FIG. 19. Same forming gonidia.
FIG. 20. Small perfect sporophore.
FIG. 21. Normal sporophore.